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Research article

Paleoparasitological analysis of human remains from a European cemetery of the 17th–19th century in Rio de Janeiro, Brazil

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ABSTRACT

Most paleoparasitological studies based on archeological sites in the New World are from pre-Columbian times. However, understanding of the introduction and spread of parasites with the arrival of European settlers and African slaves in America remains a topic for investigation. This study evaluated the presence of intestinal parasites in human remains from an archeological site of the colonial period, and compared the sensitivity of three parasitological techniques for paleoparasitological study. Samples were collected from the archeological site *Nossa Senhora do Carmo* Church, Rio de Janeiro, Brazil. Paleoparasitological examination revealed intestinal helminths in 2/17 (11.8%) individuals. *Trichuris trichiura* and *Ascaris* sp. eggs were found. The spontaneous sedimentation technique showed a greater numerical recovery of parasites, while the flotation techniques were superior in retrieving more parasite types. The study demonstrated that combining the three techniques improves the recovery of parasites in terms of number and diversity. Similar diversity of parasites to that of a previous historical archeological site suggests that the distribution of intestinal parasites was widespread in Rio de Janeiro, regardless of social status.

1. Introduction

Paleoparasitological study is becoming more widespread among researchers in paleopathology. The identification of parasites in human remains has generated information about infectious diseases and lifestyle of ancient populations, as well as helping to chart human migration routes (Araújo et al., 2008; Ferreira, 2011).

Most paleoparasitological studies in the New World have been focused on pre-Columbian times (Ferreira et al., 1980; Araújo et al., 1981; Iñiguez et al., 2003). However, the introduction and spread of parasites after the arrival of European settlers and African slaves to America remains understudied. Evidence of *Trichuris trichiura* and trichostrongylid nematode infections have been reported in archeological sites from the colonial period of Brazil, in the Minas Gerais and Piauí states (Confalonieri et al., 1981; Araújo et al., 1984). Recently, Jaeger et al. (2013) found infections with the nematodes *Trichuris* sp., *Ascaris* sp., and the cestode *Taenia* sp. in individuals buried in a cemetery in Rio de Janeiro dating to the 18th century.

The city of Rio de Janeiro, on the Atlantic coast, has a semi-humid tropical climate with an annual rainfall in the range 1000–1500 mm. The *Nossa Senhora do Carmo* Church (INSC) is located in the neighborhood of the *Largo do Paço*, in the center of Rio de Janeiro. Historically, the church was designated the Chapel Royal, and later it became the cathedral of the city. In 2007, during an architectural and artistic restoration, an archeological site was identified by the Institute of Brazilian Archeology [Instituto de Arqueologia Brasileira (IAB)]. Forty-three human burials and ossuaries were identified, dating from the 17th to 19th centuries (Dias, 2008). Bio-anthropological analysis revealed that most of the subjects were adults less than 30 years old (78%), with an equal number of men and women (Jaeger et al., 2012). A tuberculosis paleogenetic study conducted in the INSC population showed positivity for bacteria of the *Mycobacterium tuberculosis* complex in 53.1% of the individuals (Jaeger et al., 2012). Using the mitochondrial DNA, European ancestry was identified in 100% of primary burials. Considering that intestinal parasite infection is an indicator of health and hygiene conditions of a population, the aim of this study was to evaluate the presence of intestinal parasites in samples from the archeological site of the *Nossa Senhora do Carmo* Church. In addition, the sensitivity of three parasitological techniques in paleoparasitological analyses was compared.

2. Materials and methods

Sediment samples were collected directly from the pelvic region of the seventeen human bodies. All samples were derived

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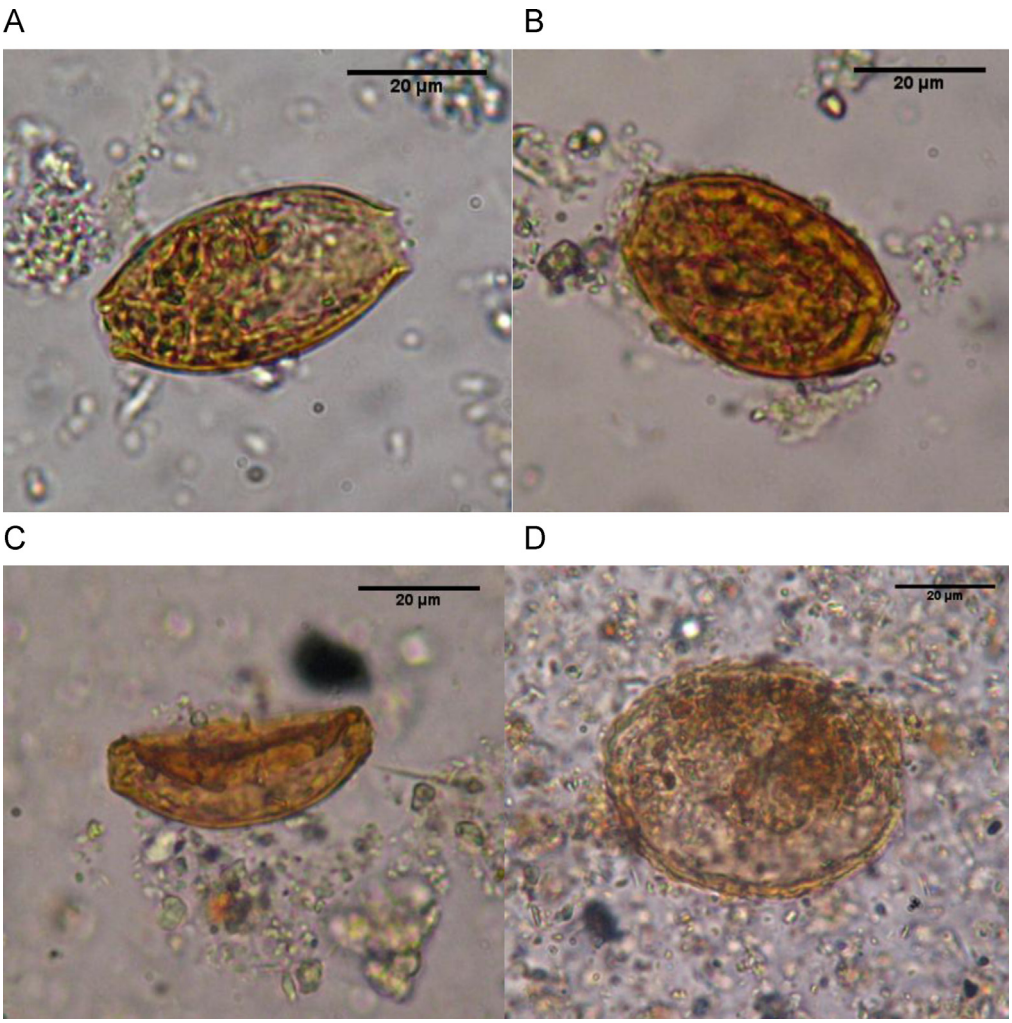

Fig. 1. Paleoparasitological results of archeological site INSC, Rio de Janeiro, Brazil. (A, B) *Trichuris* sp. eggs; (C) warped *Trichuris* sp. egg; (D) *Ascaris* sp. egg. Scale bar in the figures.

from primary burials, with the exception of burial 38, which was from secondary burial (Jaeger et al., 2012). The samples (1–2 g) were rehydrated (1:2, w/v) with 0.5% aqueous trisodium phosphate at 4 °C for 72 h. Paleoparasitological investigation was conducted using three techniques, which followed the protocol of Taglioretti et al. (2012): spontaneous sedimentation, sucrose flotation, and zinc chloride flotation. We conducted the three parasitological techniques on all samples in an effort to obtain the maximal results and for comparative purposes.

For spontaneous sedimentation (Lutz, 1919), ten slides of each sample were examined. Sucrose flotation was conducted at a sucrose density of 1.2 (Sheather, 1923, modified). At least 3 slides were examined after 15, 45, and 105 min flotation or until no further structures were recovered. The sediment samples were cleaned twice with aqueous trisodium phosphate 0.5%, and zinc chloride flotation was conducted at a density of 1.5 (Reinhard et al., 1988). At least 5 slides were examined after 10, 25, 40, 55, and 70 min flotation or until no structures were observed. A minimum of 18 slides was examined for each sample. Slides were examined under 100× and 400× magnification, and measured with an ocular micrometer. The images were made by Canon AS 650, and edited by ImageJ 1.44p (National Institutes of Health, USA). The program R (version 2.13.2) was used for descriptive statistical analyses and construction histograms.

3. Results

When the results of all three methods are combines, we find that two of seventeen (11.8%) individuals had intestinal helminthes (Table 1). A single positive sample was found when using spontaneous sedimentation (5.9%) with 10 of *Trichuris* sp. eggs recovered. Sucrose flotation and zinc chloride flotation each showed a single (5.9%) positive sample for *Ascaris* sp. and *Trichuris* sp., respectively (Table 1).

Two methods (spontaneous sedimentation and zinc chloride flotation) recovered at total of 13 *Trichuris* sp. eggs from individual 14A (Table 1 and Fig. 1A–C). Most *Trichuris* sp. eggs possessed no content or polar plugs. *Trichuris* sp. egg deformation ($n = 3$) was observed with zinc chloride flotation (Fig. 1C), and data from those samples were excluded from the descriptive analysis. *Trichuris* sp. eggs ($n = 10$) measured 40.0–47.5 µm in length and 22.5–27.5 µm in width (mean ± SD = 43.5 ± 2.48 µm L and 24.0 ± 1.84 µm W) (Fig. 2). The *Trichuris* sp. mean egg size corresponds to that established for *T. trichiura* (Confalonieri et al., 1988). A single *Ascaris* sp. egg measuring 57.5 × 45.0 µm was found in individual 38H (Fig. 1D), using the sucrose flotation technique.

4. Discussion

In the Americas, parasite infections became a major health problem after the arrival of European settlers and the slave trade.

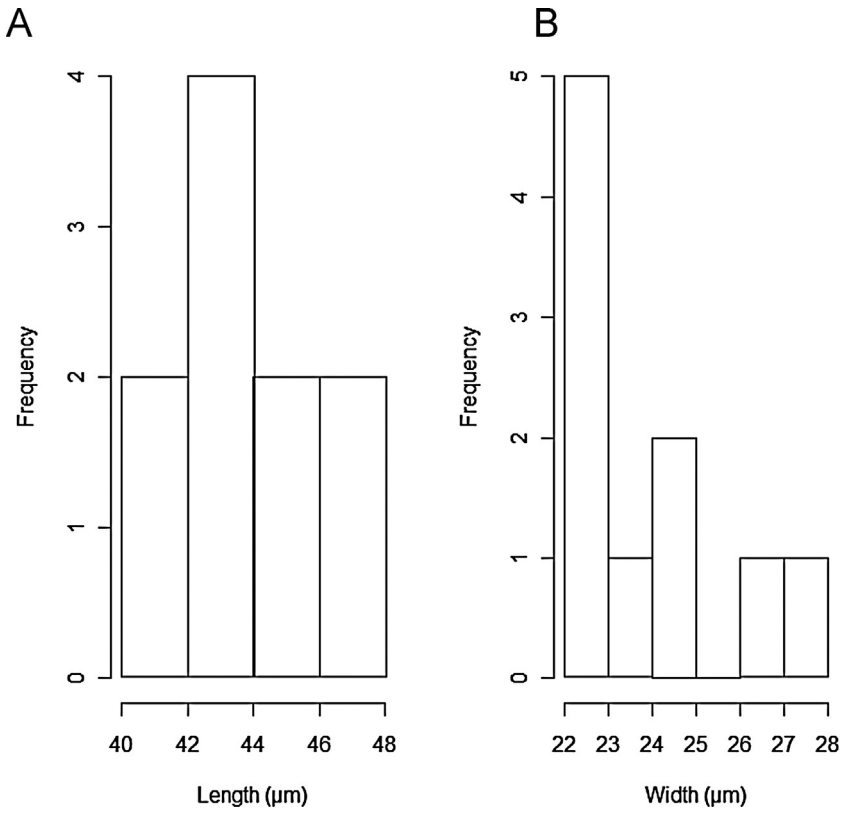

Fig. 2. Frequency histograms of (A) length and (B) width of *Trichuris* sp. eggs.

Changes in the lifestyle of the native populations, population growth and overcrowding of colonized cities, may have facilitated the transmission and spread of infectious and parasitic diseases. For this reason, and since the parasitological studies in the colonial period in Americas are scarce, the study of intestinal parasites in that historical period is necessary.

Findings of *Trichuris* sp. and *Ascaris* sp. in Brazil have been reported dating from 8000 to 7000 BP and 3490 ± 120 to 430 ± 70 BP, respectively (Goncalves et al., 2003), through the colonial period (Confalonieri et al., 1981; Araújo et al., 1984). The observation of *Trichuris* sp. and *Ascaris* sp. in the present study is in agreement with previous investigations reporting the recovery of those species in human samples dating from the Brazilian colonial period (Jaeger et al., 2013). We recently found an 80% infection for intestinal parasites in humans remains recovered in *Praça XV* cemetery. In historical times, this cemetery served as a burial place for the general population of the city, along with a considerable number of African slaves (Jaeger et al., 2013). Because the *Praça XV* samples underwent a curation process before paleoparasitological analysis, the prevalence of infection and abundance of parasites observed should be considered an underestimation. On the other hand, the individuals buried at the archeological site INSC were of European maternal ancestry, which is consistent with the clergy and more affluent population of the city (Jaeger et al., 2012). As expected, since these individuals should have lived under more healthful conditions than the general population and the African slaves, they were less parasitized than individuals from the *Praça XV* cemetery. In spite of the lower prevalence of intestinal parasites in the INSC archeological site, a similar diversity of parasites, with *Trichuris* sp. and *Ascaris* sp. was observed, suggesting that the distribution of intestinal parasites was widespread in the city, regardless of social status. A previous study showed *Mycobacterium tuberculosis* complex infection in 53.1% of individuals buried in INSC (Jaeger et al., 2012). Ten individuals analyzed in the present study (58.8%) were infected with mycobacteria, including the two individuals positive for intestinal helminths.

The presence of preserved parasites is influenced by the taphonomic conditions to which the samples have been subjected. The semi-humid tropical climate of the city affects the preservation of the parasites as well as of organic compounds. Despite this, the recovery of a reasonable egg number was possible at the INSC archeological site. Other taphonomic factors, such as proximity to the coast, the temperature variations, and possible contact with groundwater may also have influenced preservation.

Table 1
Paleoparasitological results from archeological site *Nossa Senhora do Carmo* Church, Rio de Janeiro, Brazil. (+): positive result; (−): negative result; [] number of eggs found.

Sample	Spontaneous sedimentation	Flotation		Parasites
		Sucrose	Zinc chloride	
03A	–	–	–	
04A	–	–	–	
08A	–	–	–	
12B	–	–	–	
14A	+ [10]	–	+ [3]	*Trichuris* sp.
17H	–	–	–	
18A	–	–	–	
20A	–	–	–	
22B	–	–	–	
23A	–	–	–	
25B	–	–	–	
28C	–	–	–	
30F	–	–	–	
32C	–	–	–	
33C	–	–	–	
34A	–	–	–	
38H	–	+ [1]	–	*Ascaris* sp.

Paleoparasitology usually involves morphometry to identify genus, and sometimes species, of a particular parasite. In this study, descriptive statistical analyses demonstrated that *Trichuris* sp. egg size corresponds to that established for desiccated eggs of *T. trichiura* (Confalonieri et al., 1988), the human species. Although it is not easy to assess the parasite load of an individual at time of death (Bouchet et al., 2003), the large number of eggs observed in individual 14A combined with the poor preservation of samples suggests a substantial infection by *T. trichiura*.

Concentration techniques such as sedimentation and flotation have the main objective of recovering the largest number of parasites possible from fecal samples (De Carli, 1994). In paleoparasitology, these techniques are often used because they increase the chance of finding parasitic forms in coprolites and sediment samples. Spontaneous sedimentation concentrates of the parasitic debris through deposition in an aqueous solution of lower density than eggs or other parasite forms (Fugassa, 2011). Flotation techniques are based on difference in the density between helminth eggs or protozoan cysts and the fecal material, in which the less dense organisms float to the surface of a saturated solution of suitable density (De Carli, 1994). The density of helminth eggs ranges from 1.05 and 1.15 g/mL (De Carli, 1994). Trematodes are denser than nematodes and therefore need a flotation solution of higher density than that used for nematodes (Fugassa, 2011). The disadvantage of flotation is that the solution may cause deformation or distortion of the egg wall, making identification difficult.

Paleoparasitological techniques attempt to find parasites and/or parasitic structures in archeological samples. In the present study, three parasitological techniques were employed, as in Taglioretti et al. (2012). Although Taglioretti et al. (2012) found that zinc chloride flotation was more sensitive than sucrose flotation or spontaneous sedimentation for the recovery of *Ascaris* sp., *T. trichiura*, and *Enterobius vermicularis* eggs from human fecal samples, in the present study, spontaneous sedimentation recovered the greatest number of eggs, with no deformation of the eggs compared to the other tested techniques. However, the flotation techniques recovered a greater diversity of parasite species than did spontaneous sedimentation. The difference in our results from those of Taglioretti et al. (2012) may be related to the nature of samples: modern vs. archeological sediments. Usually, paleoparasitological studies use a single concentration technique for recovering parasites, most often spontaneous sedimentation. Our observations, using each technique separately, demonstrated differing parasite infections within the population, from 5.9% prevalence with only *T. trichiura* infection (spontaneous sedimentation) to the same prevalence with only *Ascaris* sp. infection (sucrose flotation). The combined approach revealed a more complete picture of infection of the Rio de Janeiro population. Applying multiple parasite concentration techniques in paleoparasitological studies increases the recovery of parasites in both number recovered and the diversity of species.

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